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GEOCHEMICAL SIGNIFICANCE OF THE LIPID ASSEMBLAGES FROM THE *LAGUNA DE LAS MADRES* (HUELVA, SPAIN) PEAT DEPOSIT

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ABSTRACT

Vertical distributions of lipid compounds along a sediment core from *Laguna de las Madres* (Huelva, Southern Spain) peat bog was analysed by gas chromatography-mass spectrometry (GC-MS). Two peat sections taken at different depths were studied in order to get information on the structure of the trophic system and the diagenetic processes of organic matter (OM) in this depositional environment. No qualitative fluctuations downcore were observed in the patterns of the numerous alkyl series (*n*-alkanes, *n*-fatty acids, *n*-alkanols and *n*-alkan-2-ones) identified. Long-chain alkyl compounds ($>C_{24}$) typical of epicuticular waxes from herbaceous plants, predominate in these series. The presence of short-chain *n*-alkanes, bacterial fatty acids (C_{14}, C_{15}, *iso*- and unsaturated acids), des-A-triterpenoid hydrocarbons, hopanoids and some steroid ketones indicate that intense microbial reworking of the OM took place for the whole range of the geochemical record analysed in this study. Diterpenoid and triterpenoid hydrocarbons with C-backbones assigned to higher plant sources were also present, as well as hopanoids of bacterial origin. Steroid ketones and alkanols are dominated by C_{29} homologues similar, comparable to those derived from *Sphagnum* species and vascular plants. In general, the distributions of the different biomarker families were not distinct enough to be considered as diagnostic tracers of any particular input, and therefore no clear chemotaxonomic descriptors of the biodiversity changes on the palaeoecosystem along the period of the peat deposit formation could be made.

KEYWORDS

Histosols, Lipids, Biomarkers, Holocene, Hydrocarbons, Fatty acids, Peat

INTRODUCTION

Peat bog deposits were formed from peculiar paleoecosystems where the biodegradation of plant residues has been retarded due to a series of pedo-climatic and topographic factors, which lead to the progressive accumulation of OM in different evolutionary steps. From the pedological point of view peat bog deposits belong to the Histosols order, i.e. are hydromorphic soils characterised by little biological activity and a complex abiotic control of the OM transformation. For this reason, peat-formation processes are slow and the macromolecular organic components are particularly recalcitrant against biodegradation.

Due to the high performance of selective preservation processes in peat environments, the chemical molecular composition of peat deposits represent a valid record of paleoclimatic information (usually encompassing the entire Holocene period) and a useful tool in palaeoclimatic reconstructions. Different ways to take advantage from this information require the study of peat stratigraphy, as well as the pollen and macrofossils records (van Geel, 1978, Moore *et al.*, 1991; Kuder & Kruge, 1998). However, such records are not always present, or are not preserved. An alternative method is the use of organic geochemical approaches, in particular the analysis of lipid compounds derived from the plant material that contributed to the peat (Quirk *et al.*, 1984; Dehmer,

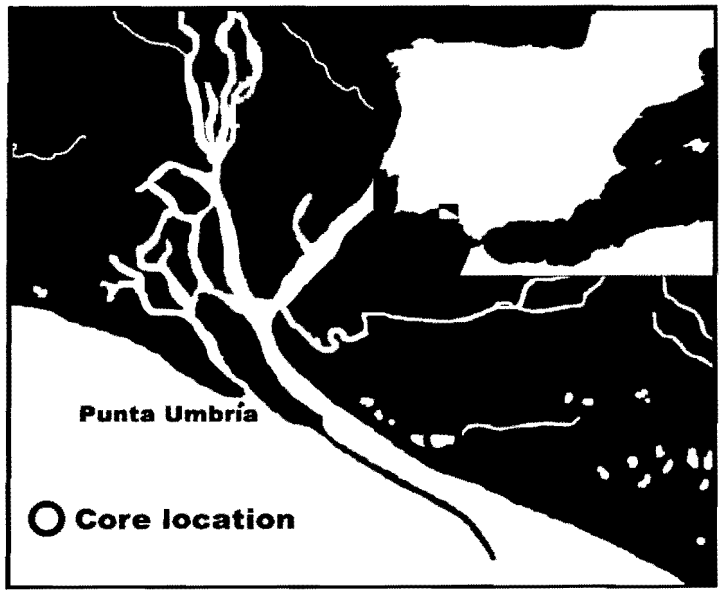

Figure 1.- Geographical location of studied area showing the core site.

1993; Lehtonen & Ketola, 1993; Farrimond & Flanagan, 1995; Ficken *et al.*, 1998; Nott *et al.*, 2000; Baas *et al.*, 2000; Duan & Ma, 2001). This is the so-called biomarker approach, which is based in the fact that lipid compounds in depositional environments may contain the basic skeletal structure and functional group details of the original source (Killops & Killops, 1993). Therefore, their occurrence can be straightforwardly associated to the main groups of organisms responsible for the accumulation and the transformation of the organic sediment which can be used to reconstruct paleoenvironmental conditions of deposition.

In this work we have used the above strategy in order to analyse the composition and diagenetic processes of OM in *Laguna de las Madres* peat-filled deposit close to the estuaries of Tinto and Odiel rivers. In particular *Laguna de las Madres'* ombrotrophic bog is considered the most meridian among the peat formations in the Northern hemisphere. This fact, together with a remarkably peculiar geomorphology (Cáceres *et al.*, 1997) defines a high-interest scenario for Mediterranean palaeoclimatic research. The biomarker data should hopefully complement previous sedimentological and palynological proxies (Menéndez-Amor & Florschutz, 1964; Stevenson, 1985; Zazo *et al.*, 1996), revealing vegetational history and possible anthropogenic interferences in this depositional environment, and all together should be correlated with climatic changes in the last 4–5 kyr.

MATERIALS AND METHODS

Sampling site

Laguna de las Madres bog (Fig. 1) has been described by IGME (1975, 1976) and Mudarra & Clemente (1978). It is a thick peat layer formation up to 5 m depth and *ca.* 30 ha representing part of mid- and the whole late Holocene *ca.* 4 000 years of peat accumulation. The microtopology of the bog is very smooth and is located between 15–20 m.a.s.l. The dominant hydrophylic species and flowering plants of the present-day vegetation have been previously described in detail (Mudarra & Clemente, 1978; Stevenson, 1985). Almendros (1981) described the agronomical and hydrophysical properties of this peat. The samples for this study were collected from an 1-m depth core sampled in a well preserved area at the margin of the lagoon. Two sections at 30–40 cm and 70 cm were taken, which were frozen until analyses.

2.2. Organic matter fractions

The peat OM was fractionated into water-soluble, lipid and humic fractions following standard procedures in humus chemistry (Almendros *et al.*, 1982; González-Vila *et al.*, 1999). Total organic carbon and organic carbon distribution into the different fractions isolated were determined by wet oxidation with potassium dichromate.

Lipid analyses

Finely ground (homogenised to 1 mm) freeze-dried peat samples (10 g) were Soxhlet extracted with a dichloromethane-methanol (2:1) mixture for 48 hours. Total lipid extracts were dehydrated with anhydrous Na_2SO_4, then filtered, desiccated under reduced pressure and saponified with 0.5 mol/L KOH/methanol for 2 hours under reflux. The neutral lipids were isolated by extraction with *n*-hexane and the acidic lipids with dichloromethane after acidification to pH 1. Neutral lipid classes were further separated into subfractions by preparative column chromatography (silica-gel: alumina 1:1, deactivated with 1% water). They were successively eluted with *n*-hexane, toluene, toluene:methanol (3:1) and methanol,

respectively. The acidic lipids and polar fractions were methylated with trimethylsilyldiazomethane and silylated with *N,O-bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) before analysis by GC (Hewlett-Packard 5730A) and GC-MS (Hewlett-Packard GCD).

The separation of the different lipid compounds was achieved with a SE-52 fused silica capillary column (30 m x 0.32 mm i.d., film thickness of 0.25 mm). The column oven temperature was programmed from 40 to 100°C at 30°C min^{-1} and then to 300°C at 6°C min^{-1}. Helium was used as carrier gas at a flow rate of 1.5 mL min^{-1}. The mass spectra were acquired with an 70 eV ionizing energy. The identification of individual compound was achieved by mass fragmentography, and also by key single ion monitoring for different homologous series, low resolution mass spectrometry and comparison with published and stored (NIST and Wiley libraries) data.

RESULTS AND DISCUSSION

Table I shows the total OM content and quantitative distribution into the different OM fractions in the two peat sections studied. It is noticeable the slight decrease with depth of both the amounts of total OM and of the different humic fractions. The relatively high concentrations in water-soluble material and lipids reflect the low decomposition degree of the peat. This is in agreement with the low content in humic colloidal fractions and its low humification quality (Almendros *et al.*, 1982).

Distribution and significance of the biomarkers identified

The slight differences in lipid contents in the different sections were not reflected in the qualitative distribution of the different lipid families identified by GC-MS. That could be interpreted as no differences in the OM input, as could correspond to abrupt vegetational, climatic or enviromental changes took place during the peat formation period.

Figure 2 shows the reconstructed ion chromatographic traces corresponding to the most abundant alkyl biomarker series identified in the methylated and silylated lipid extracts from both peat sections. Comparatively lower amounts of series of diterpenoid and triterpenoid hydrocarbons, as well as steroidal biomarkers shown in Fig. 3 were identified in addition.

The homologous series of C_{14}-C_{36} *n*-alkanes (m/z 85) with a bimodal distribution with maxima at C_{19} and C_{29} were found along the peat core (Fig. 2a). A strong odd-C number predominance in the *n*-C_{27} to *n*-C_{31} range is usually related to the occurrence of higher plants, particularly angiosperms, whereas lower molecular weight alkanes ($<C_{20}$) are frequently synthesised by microorganisms, as well as phytoplankton and benthic algae (Cranwell *et al.*, 1987). The high value for the alkane C_{31}/C_{17} ratio claims for a clear strengths of autochthonous OM inputs. Peats with a large contribution of *Sphagnum* show distributions typically dominated by C_{23} or C_{25} (Baas *et al.*, 2001). Consequently, these homologues have strong potential as source-specific indicators for *Sphagnum* contribution to ancient peat deposits. In our case, they are present but not as dominant homologues, which may indicate that both *Sphagnum* and higher plants contributed to the depositional OM.

Regular isoprenoids were also detected in the range C_{13}–C_{20} in much lower concentrations than the *n*-alkanes. It is generally assumed that the major source of these compounds is the phytol side-chain of chlorophyll. The very low value of the pristane/phytane (Pr/Ph) ratio suggests that the peat formation took place in a reducing environment (Didyk *et al.*, 1978). However, the utility of this ratio and of the pristane/C_{17} and phytane/C_{18} ratio as paleoenvironmental indicator should be taken with caution, since chromans and/or tocopherols have been found to be additional potential sources for these compounds (ten Haven *et al.*,1987; Li *et al.*, 1995).

The fatty acids series were present in the range C_{12}–C_{34} (Fig. 2b), showing unimodal distribution with maximum at C_{16}. It is commonly accepted that the

	% organic C	Lipids g/100 g sample	Water-soluble OM	Total humic extract	Humic acid (HA)	Fulvic acid (FA)	Non-extractable humin	AH/AF	Peat type (a)	%N	C/N ratio
30-40 cm	38.00	3.08	0.75	15.32	10.87	4.45	21.92	2.44	Fibric	1.87	24.3
	(100)		(1.97)	(40.3)	(28.6)	(11.7)	(57.69)				
70 cm	34.50	2.99	0.46	13.66	9.03	4.63	20.37	1.95	Hemic	1.81	26.2
	(100)		(1.33)	(39.6)	(26.2)	(13.4)	(59.05)				

In brackets, percentage of the total organic carbon
a) According to the size fractionation and the respective content in fibres (Almendros, 1981)

Table I .- General analytical characteristics and distribution of total organic matter in different organic fractions from Laguna de las Madres peat at 30-40 cm and 70 cm.

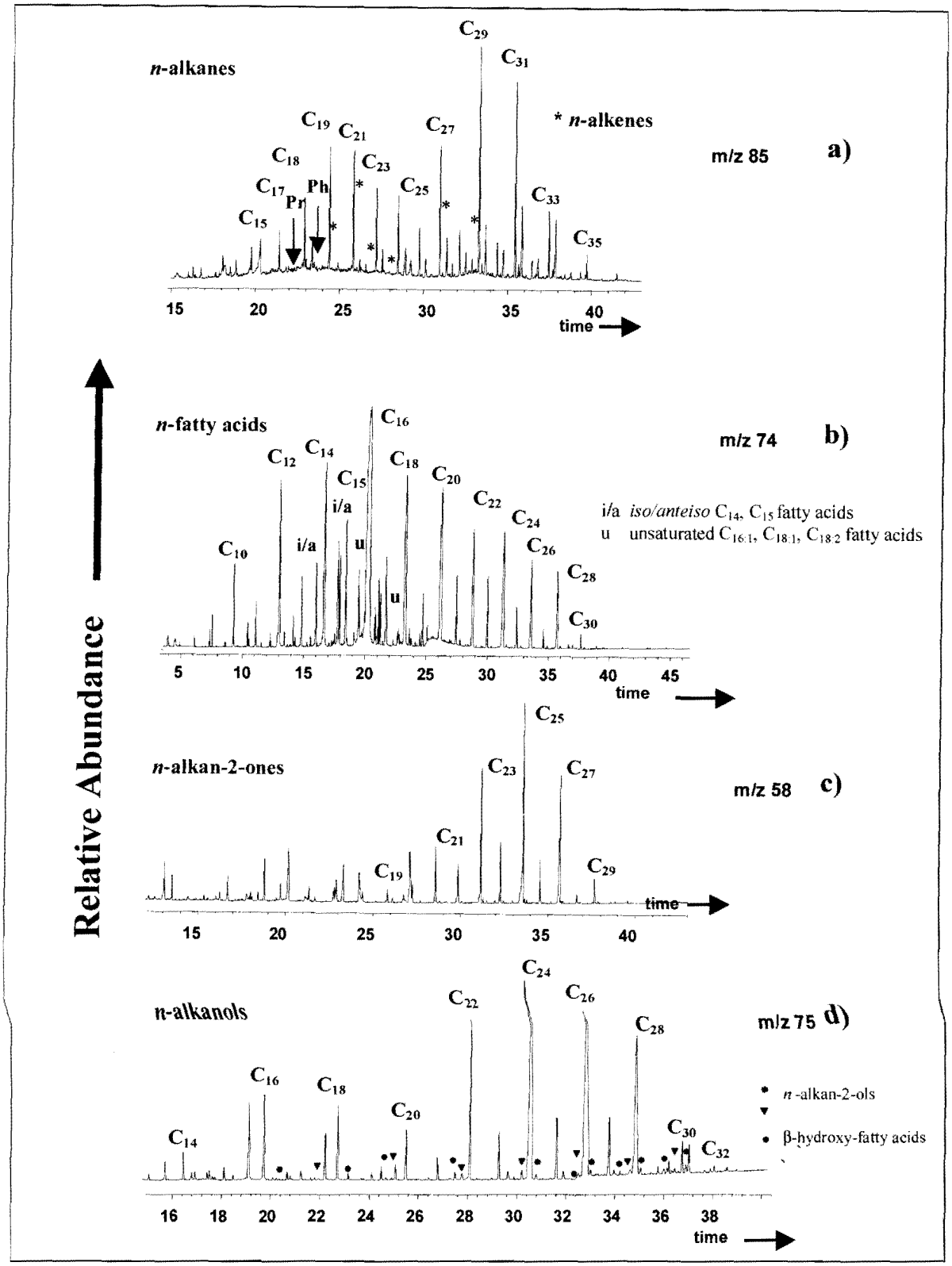

Figure 2.- Reconstructed ion chromatograms showing the traces of a) *n*-alkanes (m/z 85), b) *n*-fatty acids (m/z 74), c) *n*-alkanols (m/z 75) and d) *n*-alkan-2-ones (m/z 58) identified in lipid extract from *Laguna de las Madres* peat at 70 cm.

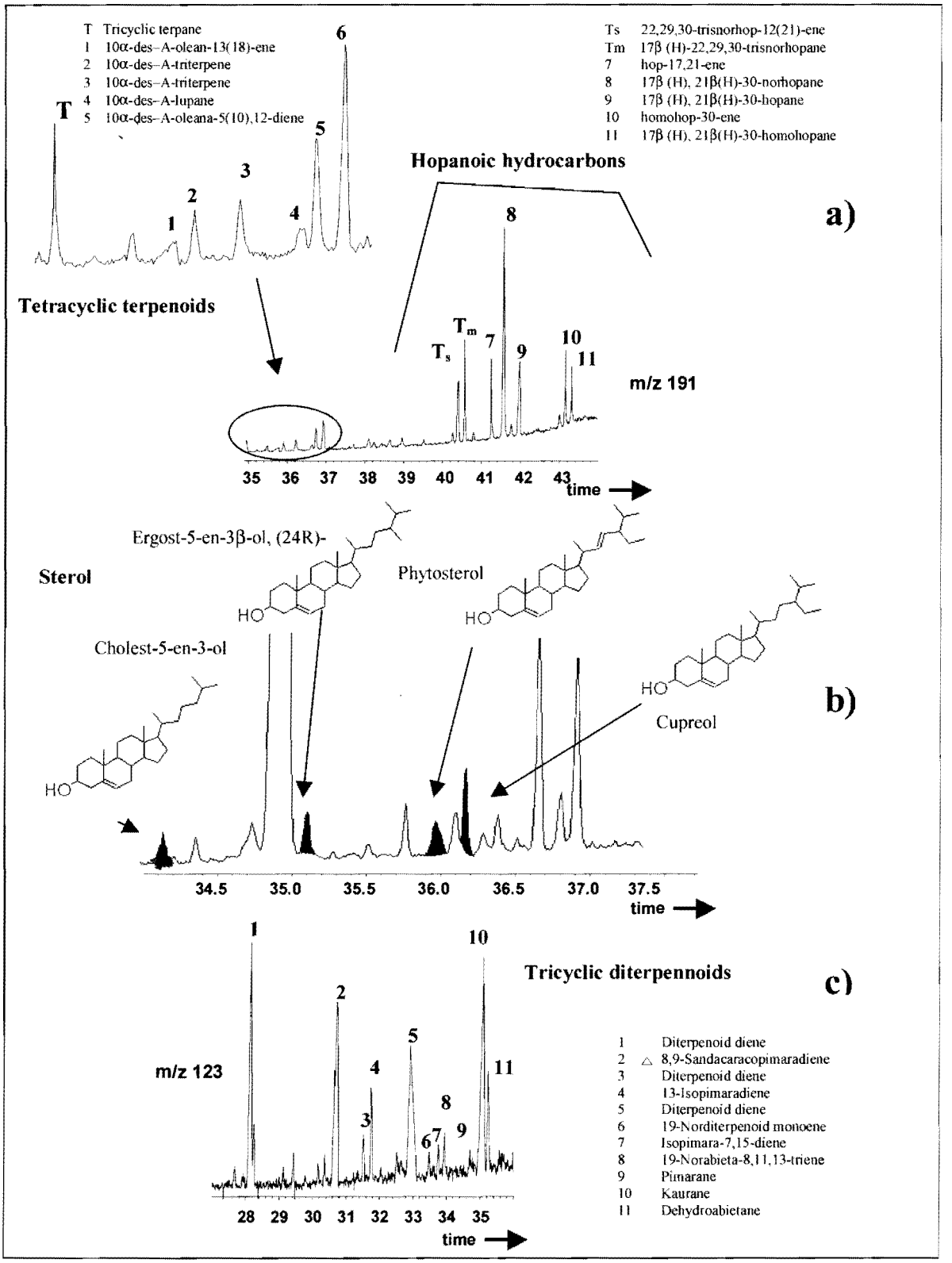

Figure 3.- Reconstructed ion chromatogram showing the traaces of a) triterpenoids (m/z 191), b) sterols and c) diterpenoids identified in lipid extract from Laguna de las Madres peat at 70 cm.

low-range fatty acids ($<C_{18}$) derive mainly from plankton and microbial biomass, whereas the longer-chain fatty acids mostly originate from plant waxes. Branched chain *iso*- and *anteiso*-acids, specific bacterial biomarkers were detected in the two lipid extracts analysed. In addition monounsaturated C_{16} and C_{18} acids and some polyunsaturated fatty acids ($C_{18:2}$ and $C_{18:3}$) typical from bacteria were also detected.

The ion traces in Fig. 2c and 2d show the distributions of *n*-alkan-2-ones (m/z 58) and *n*-alkanols (m/z 75). The molecular distribution of alkan-2-ones in the range C_{14}–C_{29} , with a predominance of odd-numbered carbon chain-lengths and a maximum at C_{25} is similar to those found in a wide variety of terrestrial and aquatic depositional environments characterised by higher plant or microbial OM inputs (Hernández *et al.*, 2001 and references therein).

A similar series of *n*-alcohols in the range C_{12}–C_{32} (Fig. 2d) with an even-to-odd carbon number predominance and maximum at C_{24} were also found in the two peat lipid extracts. The homologues $>C_{20}$ are derived from higher plants, such as herbaceous plants, whereas those $<C_{20}$ originates mainly from microbial sources (Rieley *et al.*, 1991). The ratio of homologues $<C_{20}/>C_{20}$ is also characteristic of the dominance of autochtonous productivity. Both ω- and β-hydroxy fatty acids, typical bacterial biomarkers were also present in the two peat extracts, providing an additional proof of the microbial reworking undergone by the depositional OM.

A series of des-A-triterpenoids (Fig. 3a) similar to that reported in other peat samples (Logan & Eglinton, 1994) was detected. It have been reported that these compounds originate from pentacyclic triterpenoids with oleanane or lupane skeletons, which have been found only in higher plant-derived OM (ten Haven & Rüllkotter, 1988; Logan & Eglinton, 1994) by microbial degradation processes.

Hopanoid hydrocarbons detected in the peat lipid extracts consist primarily of the 17b (H), 21b(H)-hopane serie (Fig. 3a), with C_{27}b and C_{29}bb homologues as the major components. These hopanes are considered diagenetic products of precursors synthesised by microorganisms, and are present in only immature OM (Ourisson *et al.*, 1979).

The sterols identified in the peat samples are shown in Fig. 3b. They are mainly C_{29} components, which to large extent come from higher plants (Volkman, 1986), although sterols are the most abundant free lipids in the *Sphagnum* species (Baas *et al.*, 2000).

A series of diterpenoid hydrocarbons with abietane, pimarane and kaurane skeletons occur in the peat extracts. Figure 3c shows the reconstructed ion trace of at m/z 123 in the diterpenoid region of the chromatogram. The numbers on the peaks refer to the compounds listed besides the trace. These compounds have been found to derive from many higher plant resins and tissues, and therefore have been widely used as indicator of the input of terrigenous OM.

CONCLUSIONS

No qualitative fluctuations downcore were observed in the patterns of the numerous alkyl series (*n*-alkanes, *n*-fatty acids, *n*-alkanols and *n*-alkan-2-ones) identified. Long-chain components ($>C_{24}$) typical of herbaceous origin, predominate in these series. The presence of short-chain *n*-alkanes, bacterially-derived fatty acids (C_{14}, C_{15}, *iso*- and unsaturated acids), des-A-triterpenoid hydrocarbons, hopanoids and some steroid ketones indicate that intense microbial reworking of the OM has occurred. Diterpenoid and triterpenoid hydrocarbons with skeletons assigned to higher plant sources were also present, as well as hopanoids of bacterial origin. Steroid ketones and alkanols are dominated by C_{29} homologues derived both from *Sphagnum* spp and higher plants. In general, the distribution patterns of the different biomarker families were not distinct enough to be of use in tracing a particular input. Consequently no clear relationships between chemotaxonomy and possible differences in biological habitat along the period of the peat deposit formation could be unambiguously shown.

The differences in lipid content in the core sections were not reflected in the qualitative distribution of the different lipid families as identified by GC-MS. That could means that either no differences in the OM input, reflecting any abrupt vegetational, climatic or environmental changes took place during the peat formation period, or that past climatic changes were not recorded by lipid proxies due to the strong anthropogenic influence and an intense microbial reworking of the OM in the area.

Stevenson's (1985) palynological study demonstrated the occurrence of alternant vegetation systems consisting on oak forest growing on stable soils and pine forest on unestable dune slack. This author was able to detect anthropogenic disturbances in the area as early as *c.* 4480 yrs B.P., corresponding to an Early Bronze Age cultivation of vine. Human intervention in the area has been continued untill today, being particularly aggressive in recent times when a large quantity of peat was extracted on a commercial escale during most of the second half of the XX century.

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